Original Article

Application of magnetic resonance spectroscopy in the preoperative grading of gliomas

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Abstract: This study aims to assess Proton Magnetic Resonance Spectroscopy (1H-MRS) for diagnosis and preoperative grading of cerebral gliomas. A multi point biopsy under 1H-MRS metabolism imaging guidance in 21 cerebral glioma patients was performed preoperatively to obtain the corresponding voxel tissue samples. The gliomas were graded according to MRS and results compared with routine MRI and pathology data, respectively. One hundred and sixteen tissue samples were obtained by biopsy, and the puncture target exactly matched the selected voxel. Choline/N-acetyl aspartate (Cho/NAA) ratios positively correlated with pathological grades (r=0.368, P=0.031). The Cho/NAA ratio was 2.52 with a maximum Youden index of 0.7; based on these results, MRS had 92.9% sensitivity, 71.4% specificity, and 85.7% accuracy, and was more favorable than routine plain and enhanced MRI with 64.3% sensitivity, 85.7% specificity, and 71.4% diagnosis accordance (P=0.040). MRS may contribute to accuracy improvement for grading cerebral gliomas prior to operation.

Keywords: Proton magnetic resonance spectroscopy, neuronavigation, biopsy, glioma, brain tumors

Introduction

Glioma is the most common central nervous system malignancy and arouses a great concern due to its high invasiveness, mortality and recurrence [1]. Considering the diversity in treatment and diagnosis different grades in approaches for gliomas, accurate grading is of great importance to verify prognosis and initiate the most appropriate treatment strategy for patients with gliomas. Currently, tissue samples are generally obtained by surgical resection or stereotactic biopsy and are subsequently graded pathologically; this is usually limited by grading inaccuracy with partial resection of the tumor or inappropriate biopsy specimens [2, 3]. Edward et al. [4-6] explained unconfirmed pathological diagnosis or misdiagnosis as follows: (1) traditional operation is based on the anatomical structure from MRI data, which reflect the tumor-induced destruction of the blood-brain barrier, and may result in false negatives; (2) confirming the lesions with highest malignancy is critically important due to the heterogeneity of tumor metabolism, since it may be misdiagnosed if the specimen is limited by a biopsy. Accordingly, a new approach is required for tumor grading.

The choline/N-acetyl aspartate (Cho/NAA) ratio, which comprehensively reflects the changes of compound content of tumor volume, always increases with malignancy and is quite sensitive in glioma grading. ¹H-MRS can detect the metabolism imaging of lesions by measuring the concentration alteration of particular organization metabolites and Cho/NAA, providing a new approach for the diagnosis and grading of glioma preoperatively. The current study aimed to determine an appropriate Cho/NAA value by analyzing the association between MRS and pathological grades of gliomas.

Materials and methods

Patient selection

This study was approved by the ethics committee of Huashan Hospital, Fudan University. From September 2010 to October 2011, twenty-
ty-one patients with brain tumors and MRI confirmed lesion locations but unconfirmed properties were later diagnosed with glioma by pathology postoperatively and retrospectively analyzed. There were 15 males and 6 females aged between 16 and 71 years (mean age of 48.8 ± 13.2 years); lesion volumes ranged from 24 to 62.3 cm³ (40.2 ± 7.8 cm³). The percentages of patients with lassitude, lethargy/headache and speech dysfunction were 61.9% (13/21), 39.1% (8/21) and 28.6% (6/21), respectively. All participants provided written informed consent. The glioma details are displayed in Table 1.

### Equipment and parameters

**Surgical system:** a comprehensive operating center system was used in this study, including an operating and diagnosis room (IMRIS Inc., Canada), a 3.0T MAGNETOM Verio MR system (Siemens Healthcare, Siemens AG, Germany), a Stealth Station TRA i7 Neuronavigation system (Medtronic Navigation Inc., America), and an Image post-processing workstation (Syngo Multi Modality Workplace, Siemens Healthcare, Siemens AG, Germany). The puncture appliance was Navigus System for Frameless Access (Medtronic Navigation Inc., USA).

**Magnetic resonance imaging (MRI)**

MRI T1 weighted images (TR/TE, 600/16 ms) and T2 weighted images (TR/TE, 5100/138 ms) were obtained using spin echo (SE) and fast spin echo (FSE) sequences; T1 and T2 slice thicknesses were 5-8 mm with the slice gap 1.5 mm. The complete scanning was performed in three slices. On the basis of T1 weighted images of SE sequences, MR enhanced scan images were obtained. The following indicators needed to be noted in the MRI plain scan and enhanced: (1) the situation of tumor texture and necrosis; (2) presence or absence of bleeding; (3) presence or absence of space occupying effect; (4) presence or absence of blood brain barrier damage. According to the subjective judgment of surgeons and radiologists, the cases were diagnosed as low or high grade glioma based on the status of uneven texture, necrosis, hemorrhage, occupying effect, and enhancement of tumors through MRI evaluation.

Pointed-resolved spectroscopy sequence of three dimensional multi-voxel was used in MRS, usually with the metabolites NAA, Cho, Cr, and Lip (TR/TE=1000 ms/144 ms). The voxel thickness was 10 mm with the slice gap of 2 mm. The size of field of view (FOV) depended on the lesion condition, including region of tumor enhancement, tumor edema, and normal brain tissue away from the lesion. The phasic matrix was 160×160 mm and the size of region of interest (ROI) varied from 0.5×0.5×0.5 mm³ to 1×1×1 cm³.

The ROI contained glioma enhanced area, abnormal signal area and surrounding region of tumor, and normal brain tissue can be selected as control if necessary; in addition, the selected voxel is usually in a straight line, which is convenient for needle biopsy.

**Fusion of MRS metabolic imaging and 3D navigation imaging**

All data transmitted through the local area network to the image post-processing workstation were used to automatically calculate Cho/NAA ratios with the software Spectroscopy (Syngo

### Table 1. Location, pathology classification and grades of 21 selected gliomas

<table>
<thead>
<tr>
<th>Location</th>
<th>Baseline data</th>
<th>Pathology classification</th>
<th>Grade (WHO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48.8 ± 13.2</td>
<td>4</td>
<td>II</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>15/6</td>
<td>4</td>
<td>III</td>
</tr>
<tr>
<td>Location</td>
<td>4</td>
<td>4</td>
<td>IV</td>
</tr>
<tr>
<td>Left frontal lobe</td>
<td>8</td>
<td>Oligodendroglioma</td>
<td>7</td>
</tr>
<tr>
<td>Right frontal lobe</td>
<td>2</td>
<td>Anaplastic astrocytoma</td>
<td>6</td>
</tr>
<tr>
<td>Right temporal occipital lobe</td>
<td>1</td>
<td>Glioblastoma</td>
<td>5</td>
</tr>
<tr>
<td>Left parietal lobe</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right parietal lobe</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilateral parietal lobe</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left insula</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left basal ganglia</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right thalamus</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**Fusion of MRS metabolic imaging and 3D navigation imaging**

All data transmitted through the local area network to the image post-processing workstation were used to automatically calculate Cho/NAA ratios with the software Spectroscopy (Syngo
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Multi Modality Workplace, Siemens Healthcare, Siemens AG, Germany) on the nuclear magnetic resonance system. The area with high Cho/NAA was selected to design the preoperative piercing trajectory and target. Notably, the piercing trajectory should avoid the conduction bundle and functional region as much as possible. Osirix (v.3.7.1 32-bit version, Apple Inc., USA) was used to import and read images, and record all the voxel coordinates of targets (X, Y, Z). Running in the Matlab (7.7.0 version, Mathworks Inc., America) environment, the original spectra, neuronavigation data, and voxel coordinates of biopsy targets were orderly imported to the Biopsy-NAV software, which was developed by our study group and yields a more accurate biopsy position which confirms the MRI or MRS exam (i.e. ROI). The biopsy target’s anatomic position, automatically marked in the navigation data, was then imported to the nerve navigation system, generating a fusion of MRS metabolic imaging and 3D anatomical imaging through post-processing by the workstation.

Pathological examination

All biopsy specimens fixed with 4% formaldehyde were sent to pathology department, where they were paraffin embedded, sectioned, stained with hematoxylin and detected by immunohistochemistry (EnVision). Diagnosis with H&E and immunohistochemical staining was confirmed by two neuropathology specialists according to the fourth edition of “WHO tumors of the central nervous system” published in 2007. Any discrepancy between two specialists was resolved by discussion and consultation with two additional neuropathology specialists.

Statistical analysis

All the statistical analyses were performed using SPSS 13.0 (Chicago, IL, USA). Receiver operating characteristic (ROC) curves were plotted, area under the curve (AUC) values was calculated, sensitivity and specificity was determined, and correlation analysis was performed. Comparisons among groups were executed by Wilcoxon rank sum test. P<0.05 was considered to be statistically significant.

Results

Functional imaging

The routine structure imaging of both MRI and MRS was obtained with stable spectral baselines and apparently increased Cho values and decreased NAA amounts in the concerned areas.
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Pathology diagnosis

One hundred and sixteen tissue samples were obtained from 21 patients with cerebral glioma by biopsy, and puncture targets were exactly matched to the selected voxels with a deviation from the target averaging 0.5 ± 0.45 mm. Using pathological examination as gold standard, grades I and II gliomas were considered to be low grade, and grades III and IV were categorized as high grade. A total of 7 cases were diagnosed as low grade (7/21, 33%), and 14 as high grade (14/21, 67%) accordingly. Discrepancy between biopsy pathology results and postoperative pathology data was shown in Table 2.

A small amount of bleeding at puncture site was found on iMRI imaging in 1 patient after puncture, and craniotomy was immediately performed. The contralateral myodynamia of this patient decreased after operation, but recovered after two months. No new dysneuria was found in the remaining cases. Also, no death or infection after operation was found in the participants.

Comparison between MRS and routine MRI

Eleven and ten cases were diagnosed as low and high grade gliomas, by conventional MRI scan; meanwhile, six and fifteen cases were attributed to low and high grade gliomas by MRS according to the ROC results (showed below). Importantly, pathological diagnosis confirmed 7 low and 14 high grade gliomas (Table 3).

All the cases were categorized in three groups according to pathology grade II/III/IV, with Cho/NAA values of 5.36±4.43, 2.59±2.42, and 2.07±1.99 (P<0.05), respectively, indicating a significant difference among groups. The means and variances of Cho/NAA ratios were calculated, from which the ROC curve was derived (Figure 1). A Cho/NAA cutoff of 2.52 allowed diagnosis of low or high grade pathology; the maximum Youden index was 0.7, and the area under the ROC curve was 0.725 (95% CI: 0.527-0.923). These results were compared to the gold standard with a Cho/NAA cutoff or with subjective judgment according to MRI imaging (Table 4). Considering Cho/NAA as 2.52, a significant difference was found between MRS and the conventional MRI scan (P=0.040). A typical case is presented in Figures 2-4, describing a 59-year-old male.

Discussion

Conventional and enhanced MRI can indeed provide important information for many diseases; however, high grade gliomas are easily misdiagnosed as low ones. In this current study, diagnosis sensitivity based on conventional MRI was only 64.3% (Table 4), with a considerable fraction of high grade gliomas misdiag-

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**Table 2. Pathological grades of biopsy specimens and postoperative pathology data**

<table>
<thead>
<tr>
<th>Biopsy judgment</th>
<th>Pathological grade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>II</td>
</tr>
<tr>
<td>Cancer-free</td>
<td>6</td>
</tr>
<tr>
<td>II</td>
<td>23</td>
</tr>
<tr>
<td>III</td>
<td>27</td>
</tr>
<tr>
<td>IV</td>
<td>17</td>
</tr>
</tbody>
</table>

**Table 3. Diagnoses by pathology, MRS and routine MRI**

<table>
<thead>
<tr>
<th>Pathological diagnosis</th>
<th>Routine MRI diagnosis</th>
<th>MRS diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High grade</td>
<td>Low grade</td>
</tr>
<tr>
<td>High grade</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Low grade</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>11</td>
</tr>
</tbody>
</table>

**Figure 1. ROC curve of the Cho/NAA value for tumor tissue.**

**Pathology diagnosis**

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Table 4. Sensitivity, specificity, misdiagnosis, and diagnosis accordance of MRS and routine MRI, the golden standard of "pathology examination"

<table>
<thead>
<tr>
<th>Group</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>NPVs</th>
<th>PPVs</th>
<th>DA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine MRI</td>
<td>64.3%</td>
<td>85.7%</td>
<td>54.5%</td>
<td>90.0%</td>
<td>71.4%</td>
</tr>
<tr>
<td>MRS</td>
<td>92.9%</td>
<td>71.4%</td>
<td>83.3%</td>
<td>86.7%</td>
<td>85.7%</td>
</tr>
</tbody>
</table>

NPVs: negative predictive values; PPVs: positive predictive values; DA: diagnose accordance.

In this current study which involved 116 tissue samples from 21 cerebral glioma patients, a significant difference in Cho/NAA between low and high grade gliomas was found, with the Cho/NAA ratio significantly associated with glioma grading ($r=0.368$, $P=0.01$), and this result supported the notion that the malignancy degree increases with the Cho/NAA value, in accordance with previous reports [9, 16]. Moreover, we found a Cho/NAA cutoff of 2.52 with the maximum Youden index of 0.7, indicating that MRS, with 92.9% sensitivity, 71.4% specificity, and 85.7% accuracy, is superior to routine plain and enhanced MRI ($P=0.040$). Diagnosis accuracy, especially of high grade gliomas, is crucial for treatment and prognosis.
Therefore, it is of great importance to identify a threshold value with maximum diagnostic accordance rate for glioma grading and this would need further investigation. Law and et al. [18] set a Cho/NAA threshold value of 0.75, and obtained high grade glioma diagnosis sensitivity and specificity of 96.7 and 10.0%, respectively, suggesting that lower Cho/NAA threshold is more likely to yield higher sensitivity. However, the underlying low specificity may result in excessive medical treatment. Conversely, high specificity may lead to the delay of effective treatment. Consequently, the Cho/NAA value corresponding to the maximum Youden index was selected as cut-off in this study, and although sensitivity and specificity were not favorable, this setting is more effective in distinguishing low and high grade gliomas. Indeed, an improved prediction capacity was achieved with Cho/NAA cutoff of 2.52 (maximum Youden index), in comparison with routine MRI examination. This cut-off value needs further validation in larger sample size studies.

The highlights of this study include: (1) the independently developed marking software Biopsy-NAV, whose accuracy was confirmed by iMRI scan, with a target acquisition rate of 100%; (2) the application of 3.0T MRI to collect body metabolism data of MRS with various voxels may improve image quality, reduce voxel size, ameliorate spatial and spectral resolution, and shorten acquisition time; (3) the effect of brain shift on the material may be effectively avoided by MRS metabolism imaging-guided biopsy before craniotomy.

Nevertheless, some limitations should be noted [18, 19]. $^1$H-MRS is potentially susceptible to the interference of some portion of the selected ROI close to the skull, cavernous sinus, and cerebral ventricle, which might lead to instable baseline and low spectral imaging quality. In addition, the study adopted a Cho/NAA-based univariate analysis, with Cr and Lip roles in glioma grading not discussed. Furthermore, the obtained results are based on primary clinical experience with a relatively small
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sample size; therefore, conclusions should be verified in large sample size studies.

In summary, $^1$H-MRS as a diagnostic tool, may contribute to accuracy improvement of preoperative glioma grading and needle biopsy [20].

Acknowledgements

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Disclosure of conflict of interest

None.

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References


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